OPTIMIZATION OF POLYPHENOL EXTRACTION FROM AGED TEA LEAVES USING DESIRABILITY METHODOLOGY

TỐI ƯU HÓA QUÁ TRÌNH TRÍCH LY POLYPHENOL TỪ LÁ CHÈ GIÀ BẰNG PHƯƠNG PHÁP HÀM MONG ĐỢI

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ABSTRACT

In this study, polyphenol extraction from aged tea leaves was performed by water solvent. The tea extract was analysed for the content of total polyphenols by Folin-Denis method and the antioxydant activity of this extract was determined by DPPH free radical scavenging effect. Thus polyphenol extraction was optimized by using desirability methodology with experimental order of central composite orthogonal design (CCOD). Four main effective factors namely temperature, pH, solvent/material ratio (v/m) and extraction time were studied. The statistical analysis showed that in studied range the solvent/material ratio and temperature have had significant effects on extraction yield; meanwhile antioxydant activity was influenced by temperature, pH and solvent/material ratio. The optimal condition for polyphenol extraction was as followings: extraction time 42 minutes, pH 2.9, solvent/material ratio 10/1 (v/m) and extraction temperature 77^oC. Extraction yield and antioxydant activity have reached 73.16%, 57.29%, respectively.

TÓM TẮT

Quá trình trích ly polyphenol bằng nước từ nguyên liệu lá chè xanh già đã được thực hiện. Dịch chiết chè được phân tích polyphenol tổng số theo phương pháp Folin-Denis. Hoạt tính chống oxy hóa của polyphenol được xác định theo phương pháp quét gốc tự do DPPH. Đã tiến hành tối ưu hóa quá trình trích ly polyphenol bằng phương pháp hàm mong đợi với bố trí thí nghiệm theo quy hoạch trực giao đối xứng. Bốn yếu tố ảnh hưởng chính: thời gian, pH, tỷ lệ dung môi/nguyên liệu (v/m) và thời gian trích ly đã được nghiên cứu. Các phân tích thống kê cho thấy, trong miền khảo sát: tỷ lệ dung môi/nguyên liệu và nhiệt độ có ảnh hưởng mạnh đến hiệu suất trích ly; hoạt tính chống oxy hóa chịu ảnh hưởng nhiều của các yếu tố nhiệt độ, pH và tỷ lệ dung môi/nguyên liệu. Điều kiện tối ưu cho quá trình trích ly polyphenol: thời gian trích ly 42 phút; pH 2,9; tỷ lệ dung môi/nguyên liệu 10/1 (v/m) và nhiệt độ trích ly 77^oC. Hiệu suất trích ly đạt 73,16% và hoạt tính chống oxy hóa 57,29%.

I. INTRODUCTION

Polyphenol compounds exist widely in plants and polyphenols from green tea play an improtant role as a natural antioxydant which have ability of microorganism inhibition. Green tea polyphenols have attracted much attention in research for their role in prevention of degenerative diseases such as cancer, heart deseases, diabetes, Alzheimer, Parkison, in decrease of contamination from heavy metal or radioactivity...[1-3].

Polyphenols from green tea can be extracted by various techniques including traditional extraction by water solvent, Soxhlet extraction, microwave assisted extraction and supercritical liquid extraction. Extraction yield and antioxydant activity of polyphenol obtained depend mainly on pH, solvent/material ratio, type of solvent, extraction time and temperature [4-6]. Although many new extraction techniques have been developed, traditional extraction by water solvent is still used widely due to its economical advantage.

Statistical experimental design demonstrates more satifactory and effective than other traditional methods which only allow to study one-at-a-time factor or mathematical methods because it can be used to study many variables simultaneously with less observations in saving time and costs. This method has been widely used in different research fields such as biology, food, agriculture and medicine... Desirability methodology is considered as an excellent method for multiple objective optimization of technical factors [7].

In this study, desirability methodology and CCOD were employed to optimize polyphenol extraction from aged tea leaves by water solvent.

II. MATERIALS AND METHODS

2.1 Materials

- Aged leaves of Trung Du tea picked at winter season in Phu Ho district, Phu Tho province. Tea leaves were collected, cleaned and steamed at 100^{o}C for 3-5 minutes, then dried at 50^{o}C to the moisture of 6-7%. Dried samples were kept in two-layers-polyetylen bags and stored in dry and cool place.

- Gallic acid was purchased from BDH (England); 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich (Germany).

- Chemicals, solvents are from analytical grade (China).

2.2 Analyses methods

- Quantitative analysis of the total polyphenol was carried out using Folin-Denis Method (AOAC 952.03-2000) [8].

- Antioxydant activity of polyphenol was carried out by using the scavenging effect on DPPH radicals [9]. All tea extracts prepared had the polyphenol content (equal to gallic acid) of 100 ppm.

- Tea extracts preparation:

Samples for total polyphenol determination: approximately 3g ground and dried tea leaves was weighed, then it was placed in a 250ml flask with a condenser, 150ml distilled water was added, all was adjusted 3.5 pH using HCl 1N. Extraction was in 1 hour. The crude extract was then filtrated, vacuum concentrated and adjusted to 100ml by distilled water. The tea extracts were kept in tight bottle and stored in refrigerator.

Samples for trials: approximately 3g ground and dried tea leaves was weighed, then it was placed in a 250ml flask with a condenser. The extraction was carried out in thermoregulator at defined condition of time, temperature, solvent/material ratio and pH. The crude extract was then filtrated, vacuum concentrated and adjusted to 100ml by distilled water. The tea extracts were kept in tight bottle and stored in refrigerator.

- Statistical experimental design method: the CCOD for 4 factors: time, pH, solvent/material ratio and temperature was used; each factor was carried out at 5 levels (see Table 1). This design has 27 trials including 16 trials for factorial design, 8 trials for star points and 3 trials for central points with 2 responses as following: extraction yield-Y_1 and antioxydant activity-Y_2 (see Table 2).

Table 1. The variables and their levels

Variables	Symbols	Units	Levels				
			-α	-1	0	+1	+α
Time	A	min	42	45	53	60	63
pH	B		2.1	2.5	3.5	4.5	4.9
Solvent/material	C	v/m	3	5	10	15	17
Temperature	D	^{o}C	77	80	88	95	98

Table 2. Experimental design and results

Run	A	B	C	D	Yield Y_1 (%)	Activity Y_2 (%)
1	45	2.5	5	80	43.42	47.53
2	60	2.5	5	80	42.41	46.15
3	45	4.5	5	80	41.91	45.72
4	60	4.5	5	80	38.26	44.07
5	45	2.5	15	80	88.88	59.32
6	60	2.5	15	80	87.19	54.16
7	45	4.5	15	80	84.76	44.45
8	60	4.5	15	80	80.16	38.78
9	45	2.5	5	95	35.28	27.62
10	60	2.5	5	95	38.14	28.35
11	45	4.5	5	95	36.59	26.79
12	60	4.5	5	95	36.76	27.13
13	45	2.5	15	95	88.68	40.25
14	60	2.5	15	95	90.59	37.04
15	45	4.5	15	95	87.85	26.14
16	60	4.5	15	95	87.17	22.37
17	42	3.5	10	88	55.08	40.06
18	63	3.5	10	88	54.07	36.63
19	53	2.1	10	88	52.58	38.13
20	53	4.9	10	88	48.85	26.46
21	53	3.5	3	88	22.08	41.23
22	53	3.5	17	88	89.46	46.25
23	53	3.5	10	77	64.23	55.16
24	53	3.5	10	98	63.37	29.54
25	53	3.5	10	88	48.08	41.03
26	53	3.5	10	88	48.43	41.42
27	53	3.5	10	88	48.88	41.83

2.3 Statistical analysis

The statistical software Design-Expert 7.1 (Stat-Ease, Inc., Minneapolis, USA) was used for regression analysis of experimental data, to plot response surface and to optimize by desirability methodology. ANOVA was used to estimate the statistical parameters.

III. RESULTS AND DISCUSSION

3.1 Study on the influence factors on polyphenol extraction

Extraction yield and polyphenol activity depended on many factors such as: time, pH, solvent/material ratio and extraction temperature [4-6]. Therefore in our study, each single factor: extraction time (figure 1a), pH (figure 1b), solvent/material ratio (figure 1c) and temperature (figure 1d) was studied in order to find out its effect on extraction yield and antioxydant activity of polyphenol and based on that to choose the value range of each factor for optimization.

Fig. 1 shows that extraction yield and activity of polyphenol depended on all 4 factors. We decided to choose value ranges of each factor as followings: extraction time (45÷60min), pH (2.5÷4.5), solvent/material ratio (5/1÷15/1, v/m) and extraction temperature (80÷95°C).

3.2 Model building

The CCOD for 4 factors and results as shown in Table 2. The responses: yield and antioxydant activity were fitted with a second-order polynomial equations of four factors. The significance of model and coefficients controlled by regression analysis (Table 3a, 3b). F-values of two models are 6902.52 (Y_1), 680.58 (Y_2) in turn showed that all two models are completely significant of 99.99% ($p<0.0001$) confidence level. The p-value less than 0.05 indicates that the coefficient is significant, so in the Y_1 model all coefficients were also significant. Meanwhile, in Y_2 model only interactive coefficients AB, BD, CD were insignificant, but they were remained in the model for optimization. F-value for "lack of fit" of Y_1 model and Y_2 model were 0.76 ($p=0.6906$) and 1.72 ($p=0.4237$), respectively. They showed that the two models were fit with experiment. In addition, the coefficients of determination (R^2) of the two models 0.9999 and 0.9987 in turn showed that the models were well matched with experiment designed. The extraction yield and antioxydant actitvity of polyphenol are performed in second-degree model as followings:

$$Y_1 = 48.82 - 0.41A - 1.32B + 23.89C - 0.36D - 0.68AB - 0.21AC + 0.95AD - 0.60BC + 0.78BD + 2.03CD + 2.74A^2 + 0.81B^2 + 3.34C^2 + 7.36D^2$$

$$Y_2 = 40.85 – 1.23A – 4.07B + 1.81C - 9.04D - 0.11AB - 0.99AC + 0.50AD – 3.32BC + 0.21BD + 0.17CD – 1.04A^2 – 4.06B^2 + 1.66C^2 + 0.97D^2$$

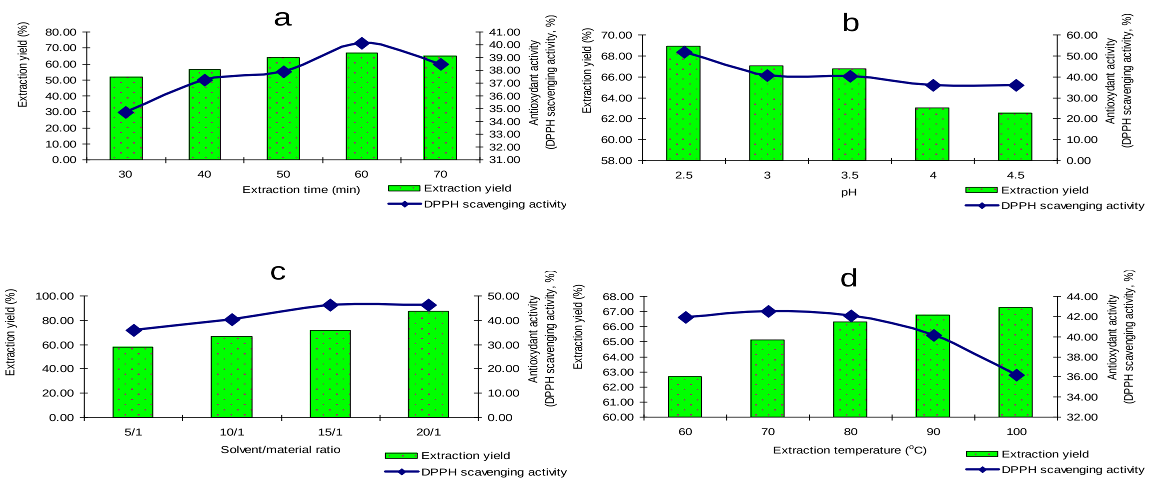

Fig. 1 Influence of factors on polyphenol extraction

Table 3a. Regression analysis of extraction yield (Y_1)

Source	Mean square	F-value	p value (prob>F)
Model	884.83	6902.52	< 0.0001
A	3.30	25.71	0.0003
B	34.86	271.95	< 0.0001
C	11414.63	89045.32	< 0.0001
D	2.55	19.92	0.0008
AB	7.33	57.19	< 0.0001
AC	0.74	5.74	0.0338
AD	14.46	112.79	< 0.0001
BC	5.84	45.59	< 0.0001
BD	9.75	76.06	< 0.0001
CD	66.14	515.94	< 0.0001
A^2	66.26	516.92	< 0.0001
B^2	5.80	45.23	< 0.0001
C^2	98.31	766.88	< 0.0001
D^2	476.99	3720.96	< 0.0001
Lack of fit	0.12	0.76	0.6906

Table 3b. Regression analysis of antioxydant activity (Y2)

Source	Mean square	F-value	p-value (prob>F)
Model	173.95	680.58	< 0.0001
A	30.31	118.58	< 0.0001
B	331.90	1298.56	< 0.0001
C	65.70	257.06	< 0.0001
D	1633.02	6389.22	< 0.0001
AB	0.19	0.73	0.4090
AC	15.70	61.43	< 0.0001
AD	3.95	15.46	0.0020
BC	176.16	689.22	< 0.0001
BD	0.68	2.68	0.1276
CD	0.45	1.74	0.2114
A^2	9.45	36.96	< 0.0001
B^2	145.40	568.86	< 0.0001
C^2	24.39	95.43	< 0.0001
D^2	8.26	32.32	0.0001
Lack of fit	0.27	1.72	0.4237

Considering in turn the effect of each factor (when other factors are remained in central level) on extraction yield (Figure 2a) and antioxydant activity of polyphenol (Figure 2b), it showed that the solvent/material ratio (C) and extraction temperature (D) have had a significant role on the yield obtained; meanwhile extraction time (D) and pH (B) took an important role on antioxydant activity. This can be shown clearly in the response surface of Y_1 function (Figure 3a) and Y_2 function (Figure 3b).

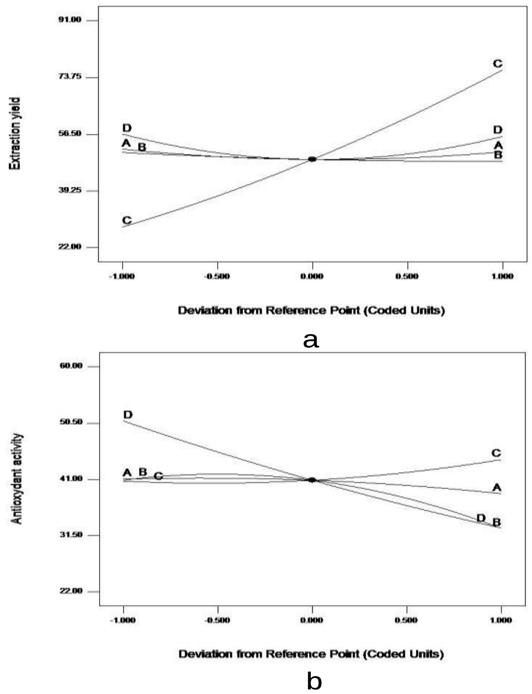

Fig. 2 Influence of factors on polyphenol extraction

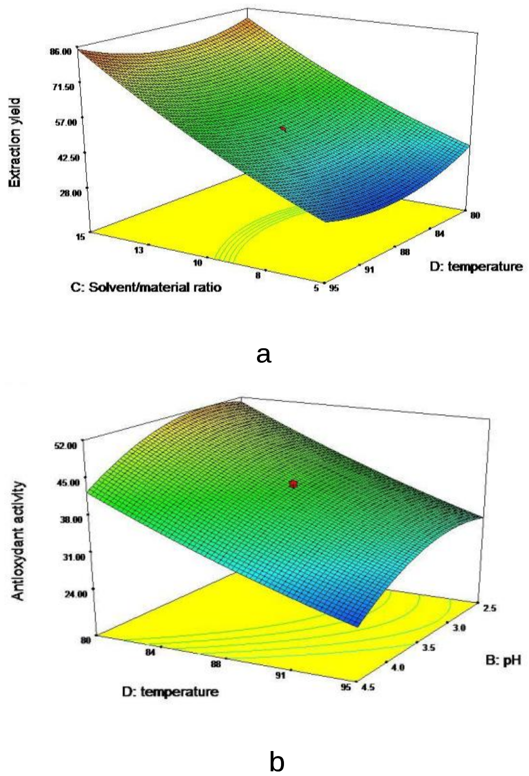

Fig. 3 Response surface plot for extraction yield and antioxydant activity of polyphenol

3.3 Optimization of the polyphenol extraction

The most important objective of polyphenol extraction process from aged tea leaves is to get the highest extraction yield and antioxydant activity. To this point, optimization by using algorism of fastened targets according to desirability methodology invented by Derringer and Suich was applied [7]. The results after using Design-Expert 7.1 software were as followings: extraction time 42 minutes, pH 2.9, solvent/material ratio 10/1 and extraction temperature 77^{o}C; in this condition, extraction yield and antioxydant activity have reached to 73.16% and 57.29%, respectively. The extraction yield, antioxydant activity and combined objective have obtained 75%, 95% and 93% desirability of objective proposed, respectively (Figure 4).

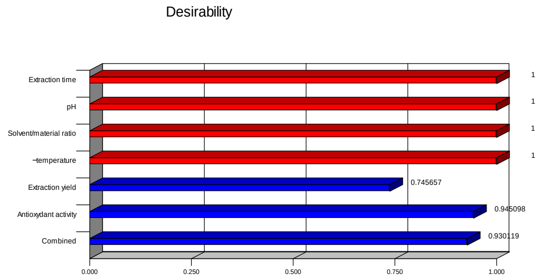

Fig. 4 Responsible desirability level

IV. CONCLUSIONS

With statistical experimental design using response surface and desirability methodology, optimal condition for polyphenol extraction by water solvent was determined as followings: extraction time 42 minutes, pH 2.9, solvent/material ratio 10/1 (v/m) and extraction temperature 77^{o}C. The extraction yield obtained 73.16% and antioxydant activity obtained 57.29%.

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